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(71) Applicants *(for all designated States except US)*: SOCIETE DE CONSEILS DE RECHERCHES ET D'APPLICATIONS SCIENTIFIQUES S.A. [FR/FR]; 51-53, rue du Docteur-Blanche, F-75016 Paris (FR). INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE [FR/FR]; 101, rue de Tolbiac, F-75654 Paris Cédex 13 (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: WDZIECZAK-BAKALA, Joanna [FR/FR]; 9, avenue de Saint-Mande, F-75012 Paris (FR). ROUSSEAU-PLASSE, Anne [FR/FR]; 18, rue Pottier, F-78150 Les Chesnay (FR). LENFANT, Maryse [FR/FR]; 18, rue des Chataigniers, F-91190 Gif-sur-Yvette (FR).

(74) Agent: COCKBAIN, Julian; Frank B. Dehn & Co., 179 Queen Victoria Street, London EC4V 4EL (GB).

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(57) Abstract

A method of promoting regeneration of hemopoietic cells in a subject undergoing chemotherapy or radiotherapy, which method includes administering to the subject an angiotensin-converting enzyme inhibitor, the amount being effective to reduce the proliferation of hemopoietic cells during or after said chemotherapy or radiotherapy.

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PROTECTION OF HEMOPOIETIC CELLS DURING CHEMOTHERAPY OR RADIOTHERAPY

Background of the Invention

Acute and chronic bone marrow toxicities are the major limiting factors in the treatment of cancer. They are both related to (1) a decrease in the number of hemopoietic cells (e.g., pluripotent stem cells and other progenitor cells) caused by both a lethal effect of cytotoxic agents or radiation on these cells and by differentiation of stem cells provoked by a feed-back mechanism induced by the depletion of more mature marrow compartments and (2) a reduction in self-renewal capacity of stem cells, which is also related to both direct (mutation) and indirect (aging of stem cell population) effects. Acute myelosuppression as a consequence of cytotoxic chemotherapy is well recognized as a dose-limiting factor in cancer treatment. Although other normal tissues may be adversely affected, bone marrow is particularly sensitive to the proliferation-specific treatment such as chemotherapy or radiotherapy.

Angiotensin I-Converting Enzyme (ACE) (peptidyl dipeptidase A, kininase II, EC 3.4.15.1) is a zinc-dipeptidyl carboxypeptidase, whose major physiological function is to cleave the C-terminal dipeptide of angiotensin I (AI) leading to the production of the potent vasoconstrictor angiotensin II (AII) (Skeggs, LT, et al., J. Exp. Med. 103:295 (1956)). As shown by *in vitro* assays, this function might be enhanced through an ACE dependent inactivation of the vasodilator bradykinin, which is inactivated by the characteristic C-terminal dipeptidic cleavage (Yang, HYT, et al., Biochim. Biophys. Acta 214:374 (1970)). These activities explain the predominant role of ACE in the regulation of blood pressure and the extensive use of ACE inhibitors in antihypertensive treatment.

Summary of the Invention

The present invention relates to a method of promoting regeneration of hemopoietic cells in a subject undergoing chemotherapy or radiotherapy.

In one aspect, the present invention features a method of promoting regeneration of hemopoietic cells in a subject undergoing chemotherapy or radiotherapy, the method comprising administering to the subject an angiotensin-converting enzyme inhibitor, the amount being effective to reduce the proliferation of hemopoietic cells during or after the chemotherapy or radiotherapy.

In one embodiment, the method further comprises administering to the subject a hemopoiesis growth factor after the administration of angiotensin-converting enzyme inhibitor and chemotherapy or radiotherapy, the amount being effective to stimulate the proliferation or differentiation of hemopoietic cells.

As will be further discussed below, by "chemotherapy" is meant a process of killing proliferating cells using a cytotoxic agent and by "radiotherapy" is meant a process of killing proliferating cells by using irradiation. The subject may intentionally or unintentionally be exposed to said cytotoxic agents or irradiation. The phrase "during the chemotherapy" or "during the radiotherapy" above refers to the period in which the effect of the administered cytotoxic agent or irradiation lasts. On the other hand, the phrase "after the chemotherapy" or "after the radiotherapy" above is meant to cover all situations in which an angiotensin converting enzyme inhibitor is administered after the administration of a cytotoxic agent or irradiation regardless of any prior administration of the same or another angiotensin converting enzyme inhibitor and also regardless of the persistence of

the effect of the administered cytotoxic agent or irradiation.

By angiotensin converting enzyme inhibitor ("ACE inhibitor") is meant a compound which is capable of inhibiting the binding to ACE of its substrates. Examples of ACE inhibitors include, but are not limited to, lisinopril, captopril, enalapril, and fosinopril.

Examples of a hemopoiesis growth factor which can be used to practice the above method include, but are not limited to, cytokines or agonists thereof, such as natural, synthetic or modified interleukins (e.g., IL-1, IL-3, IL-6, IL-11, or its agonist), G-CSF (i.e., granulocyte colony-stimulating factor), GM-CSF (i.e., granulocyte/ macrophage-colony stimulating factor), erythropoietin, stem cell factor, and leukemia inhibitory factor.

In one embodiment of this method, the subject is undergoing chemotherapy with a cytotoxic agent. By "cytotoxic agent" is meant an agent which kills proliferating cells, e.g., tumor cells, virally infected cells, or hemopoietic progenitor cells. Examples of a cytotoxic agent which can be used to practice the above method include, but are not limited to, cyclophosphamide, taxol, daunorubicine, 5-fluorouracil, adriamycin, cisplatinum, methotrexate, cytosine arabinoside, mitomycin C, prednisone, vindesine, carboplatinum, vincristine, and an agonist of any of the above compounds. A cytotoxic agent can also be an antiviral agent, e.g., AZT (i.e., 3'-azido-3'-deoxythymidine). In another embodiment of this method, the subject is undergoing radiotherapy. Note that the terms "chemotherapy" and "radiotherapy" used herein refer to the process of killing proliferating cells by administration of a cytotoxic agent or by irradiation. Radiotherapy includes only exposure to potentially harmful irradiation.

The ACE inhibitor can be administered prior to, during, or subsequent to the chemotherapy or radiotherapy (i.e., prior to, during, or subsequent to the administration of a cytotoxic agent or irradiation). It is preferred that a hemopoiesis growth factor be administered subsequent to the administration of the ACE inhibitor. Note that the timing of when to administer an ACE inhibitor depends on the half life of the compound, the duration of its inhibitory activity, the administration route, etc., as well as the conditions of the chemotherapy or radiotherapy (e.g., the half life of the cytotoxic agent which is used in chemotherapy).

The effective amount of the ACE inhibitor or growth factor used to practice the present invention varies depending upon the manner of administration, the age and the body weight of the subject, and the condition of the subject to be treated. Ultimately, it will be decided by the attending veterinarian or physician. Any such amount of the ACE inhibitor or the growth factor as determined by the attending veterinarian or physician is referred to herein as "effective amount".

Also note that the ACE inhibitor and the hemopoiesis growth factor may be administered by any route appropriate to the condition being treated. Preferably, it is administered orally or injected into the bloodstream of the subject being treated. However, it will be readily appreciated by those skilled in the art that the route, such as intravenous, subcutaneous, intramuscular, intraperitoneal, nasal, oral, etc., will vary with the condition being treated and the activity of the compound being used. Note that continuous administration using a subcutaneous infusion pump may be desirable when the compound to be used has a rather short half life or lacks

long-lasting activity. Conversely, single or intermittent administration is acceptable or even preferable when the factor to be used has a long half life or long-lasting activity.

The ACE inhibitor or the growth factor may be conveniently presented as an ingredient of a pharmaceutical composition in unit dosage form according to any of the methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient(s) into association with the carrier which constitutes one or more accessory ingredients. In general, the formulations for tablets or powders are prepared by uniformly and intimately blending the active ingredient with finely divided solid carriers, and then, if necessary as in the case of tablets, forming the product into the desired shape and size.

In one embodiment, the method further comprises administering a hemopoiesis inhibitory factor. The hemopoiesis inhibitory factor may be administered prior, after, or during the administration of the ACE inhibitor. If a hemopoiesis growth factor is also administered, the hemopoiesis inhibitory factor should be administered prior to the administration of the growth factor. Examples of a hemopoiesis inhibitory factor which can be used to practice the above method include, but are not limited to, a transforming growth factor, an interferon, a macrophage inflammatory protein, a tumor necrosis factor, pEEDCK (i.e., pyroGlu-Glu-Asp-Cys-Lys), AcSDKP (i.e., N-Acetyl-Ser-Asp-Lys-Pro), and an agonist of any of the above factors. Using a transforming growth factor as an example, by "agonist" is meant an analog (with one or more modifications) or a fragment of that factor with the same desired activity, i.e., inhibiting the proliferation of hemopoietic cells.

Other features and advantages of the present invention will be apparent from the following drawings and description of the preferred embodiments, and also from the appending claims.

Detailed Description of the Invention

It is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Also, all publications, patent applications, patents, and other references mentioned herein are incorporated by reference.

Examples of angiotensin-converting enzyme (ACE) inhibitors include sulfhydryl-containing inhibitors (e.g., captopril, fentiapril, pivalpril, zefenopril, ano-alacepril), dicarboxyl-containing ACE inhibitors (e.g., enalapril, lisinopril, benzepril, indolapril, pentopril, indalapril, and ciazlapril), and phosphorus-containing ACE inhibitors (e.g., fosinopril). Jackson, et al., Renin and Angiotensin in Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th ed., eds. Hardman, et al. (McGraw Hill, 1996).

The hemopoiesis growth factor which can be used to practice this invention is a compound capable of stimulating the proliferation of hemopoietic cells, such as cytokines. Preferred cytokines include interleukins, GM-CSF, and G-CSF. E.g., see R. Van Furth (ed.), Hemopoietic Growth Factors and

Mononuclear Phagocytes (Karges 1993) and Moore, M. article cited in the preceding paragraph.

Interleukin-1 (IL-1) has shown dramatic hemopoietic protective and restorative effects against lethal doses of irradiation either alone (Neta, R. et al., J. Immunol. 136:2483, 1986) or in concert with bone marrow transfer (Oppenheim, J.J., et al., Blood 74:2257, 1989), and against chemotherapeutic drugs such as cyclophosphamide (Castelli, M.P., et al., J. Immunol. 140:3830, 1988; Futami, H., et al., J. Immunol. 145:4121, 1990; Fibbe, W.E., et al., Exp. Hematol. 17:805, 1989). GM-CSF and G-CSF have also been found to have marked restorative effects after irradiation (Tanikawa, S., et al., Exp. Hematol. 17:883, 1989; Schuening, F.G., et al., Blood 74:1308, 1989) or treatment with chemotherapeutic drugs both in preclinical models (Moore, M.A.S., et al., Proc. Natl. Acad. Sci. USA 84:7134, 1987; Welte, K., et al., J. Exp. Med. 165:941, 1987), as well as in human trials (Steward, W.P., et al., Cancer Treat. Rev. 17:77, 1990; Gianni, A.M., et al., J. Clin. Oncol. 8:768, 1990).

For examples of hemopoiesis inhibitory factors, see Thierry, et al., J. Med. Chem. 33:2122 (1990); Robinson, et al., Stem Cells 11:422 (1993); Paukovits, et al., Blood 81:1755 (1993); and Moore, et al., Blood 78(1):1 (1991) When the method of this invention is applied to chemotherapy, the cytotoxic agent which can be used include cyclophosphamide, taxol, daunorubicine, 5-fluorouracil, adriamycin, cisplatinum, methotrexate, cytosine arabinoside, mitomycin C, prednisone, vindesine, carboplatinum, and vincristine. The cytotoxic agent can also be an antiviral compound which is capable of destroying proliferating cells. For a general discussion of cytotoxic agents used in

chemotherapy, see Sathe, M. et al., Cancer Chemotherapeutic Agents: Handbook of Clinical Data (1978).

The method of this invention can also be applied to radiotherapy, which may comprise of either ionizing waves or particles. Examples of ionizing waves include x-rays and gamma rays. Examples of ionizing particles include alpha rays, beta rays, neutrons, electrons, and protons. Radiotherapy may be administered externally or internally. Examples of external radiotherapy include x-ray units, gamma ray units, electron beams, and neutron beams. Internal radiotherapy includes both sealed and unsealed sources. Examples of sealed sources include cobalt beam units, caesium beam units, strontium applicators, yttrium rods or pellets, gold grains, or radium, cobalt or caesium needles or tubes. Examples of unsealed sources include iodine, phosphorous, gold, and yttrium. See Walter, J., Cancer and Radiotherapy (Churchill Livingstone 1977); Ed. N.J. McNally, The Scientific Basis of Modern Radiotherapy (British Institute of Radiology 1989); and Franz Buschke and Robert G. Parker, Radiation Therapy in Cancer Management (Grove & Stratton 1972).

In one embodiment of this invention, the subject undergoes repeated cycles of treatment according to the method of this invention. Preferably, a subsequent cycle commences only after the administration of the hemopoiesis growth factor has been terminated and the subject's blood cell counts (e.g., white blood cell count) have returned to a therapeutically acceptable level (as determined by the attending veterinarian or physician), permitting the repeated chemotherapy or radiotherapy. In another embodiment, the subject is exposed to a hazardous level of irradiation.

For obvious reasons, the method of this invention can also be applied to treatment in which radiotherapy and chemotherapy are performed in conjunction.

Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. The following specific example is, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

ASSAY

CD1 male mice (Charles River Laboratory, Wilmington, MA) were exposed to 2 Gy Whole-Body γ-irradiation (CIS Bio International IBL 437C ^{137}Cs γ-radiation source, Gif-Sur-Yvette, France; dose rate: 4.66 Gy/min) and received one hour later an i.p. injection of lisinopril (10 mg/kg body weight) in saline vehicle. Appropriate saline injected controls were used. At specific times after irradiation, mice were sacrificed by cervical dislocation, the thoracic cavity was rapidly entered, the heart punctured, and blood draining into the thoracic cavity was removed using a Pasteur pipette. Blood was immediately collected on lithium heparin at 4°C and centrifuged at 3000 rpm for 15 min., and plasma was removed, aliquoted, and frozen (-20°C).

The *in vitro* high proliferative potential colony-forming cell assays (HPP-CFC) were carried out as described previously (McNiece, I., et al., Int. J. Cell Cloning 8:146 (1990); Robinson, S., et al., J. Anat. 174:153 (1991)). Bone marrow cells from both control saline treated and lisinopril treated mice (2.10^4 cells/ml) as well as both irradiated saline treated and irradiated and lisinopril treated mice (4.10^4 cells/ml), in medium supplemented with 20% horse serum and 0.3% melted agar, were aliquoted over a

0.5% agar underlayer, supplemented with WEHI and L929 conditioned medium used as a source of interleukin 3 and macrophage colony stimulating factor. Cultures were incubated for 14 days at 37°C, under 5% CO_2 in air fully humidified atmosphere, and HPP-CFC derived colonies (>0.5 mm) in the cultures were counted. The proportion of HPP-CFC in S phase was determined by incubating cells with cytosine arabinoside (250 µg/ml) for 1 hour prior to plating.

A mean of between 10-20% of HPP-CFC in S phase was observed during the first 12 hours following irradiation of the mice, whereas a significant increase of the proportion of cells in DNA synthesis (33% in S phase) occurred after 24 hours. The proportion of HPP-CFC in S phase was still 30% after 72 hours. When lisinopril was administered at one hour following irradiation, the proportion of hematopoietic stem cells in S phase determined after 24 hours was identical to the value obtained in control saline mice.

Other Embodiments

The foregoing description has been limited to specific embodiments of this invention. It will be apparent, however, that variations and modifications may be made to the invention, with the attainment of some or all of the advantages of the invention. Such embodiments are also within the scope of the following claims.

What is claimed is:

Claims

1. A method of promoting regeneration of hemopoietic cells in a subject undergoing chemotherapy or radiotherapy, said method comprising administering to the subject an angiotensin-converting enzyme inhibitor, said amount being effective to reduce the proliferation of hemopoietic cells during or after said chemotherapy or radiotherapy.

2. The method of claim 1, wherein said method further comprises administering to the subject a hemopoiesis growth factor after said administration of angiotensin-converting enzyme inhibitor and chemotherapy or radiotherapy, said amount being effective to stimulate the proliferation or differentiation of hemopoietic cells.

3. A method of claim 1, wherein said angiotensin-converting enzyme is administered during chemotherapy or radiotherapy.

4. A method of claim 2, wherein said angiotensin-converting enzyme is administered during chemotherapy or radiotherapy.

5. The method of claim 1, wherein said angiotensin-converting enzyme inhibitor is captopril, fentiapril, pivalpril, zefenopril, ano-alacepril, enalapril, lisinopril, benzepril, indolapril, pentopril, indalapril, ciazlapril, and fosinopril.

6. The method of claim 5, wherein the subject is undergoing chemotherapy with a cytotoxic agent, wherein said cytotoxic agent is cyclophosphamide, daunorubicine, taxol,

5-fluorouracil, adriamycin, cisplatinum, methotrexate, cytosine arabinoside, mitomycin C, prednisone, vindesine, carboplatinum, AZT, or vincristine.

7. The method of claim 5, wherein the subject is undergoing radiotherapy.

8. A method of claim 1, wherein said method further comprises administering to said subject a hemopoiesis inhibitory factor.

9. The method of claim 3, wherein said angiotensin-converting enzyme inhibitor is captopril, fentiapril, pivalpril, zefenopril, ano-alacepril, enalapril, lisinopril, benzepril, indolapril, pentopril, indalapril, cizalapril, and fosinopril.

10. The method of claim 9, wherein the subject is undergoing chemotherapy with a cytotoxic agent, wherein said cytotoxic agent is cyclophosphamide, daunorubicine, taxol, 5-fluorouracil, adriamycin, cisplatinum, methotrexate, cytosine arabinoside, mitomycin C, prednisone, vindesine, carboplatinum, AZT, or vincristine.

11. The method of claim 9, wherein the subject is undergoing radiotherapy.

12. A method of claim 3, wherein said method further comprises administering to said subject a hemopoiesis inhibitory factor.

13. The method of claim 2, wherein said hemopoiesis growth factor is a cytokine.

14. The method of claim 13, wherein said angiotensin-converting enzyme inhibitor is captopril, fentiapril, pivalpril, zefenopril, ano-alacepril, enalapril, lisinopril, benzepril, indolapril, pentopril, indalapril, ciazlapril, and fosinopril.

15. The method of claim 14, wherein said hemopoiesis growth factor is G-CSF or GM-CSF.

16. The method of claim 15, wherein the subject is undergoing chemotherapy with a cytotoxic agent, wherein said cytotoxic agent is cyclophosphamide, daunorubicine, taxol, 5-fluorouracil, adriamycin, cisplatinum, methotrexate, cytosine arabinoside, mitomycin C, prednisone, vindesine, carboplatinum, AZT, or vincristine.

17. The method of claim 15, wherein the subject is undergoing radiotherapy.

18. A method of claim 3, wherein said method further comprises administering to said subject a hemopoiesis inhibitory factor prior to said administration of said hemopoiesis growth factor.

19. The method of claim 4, wherein said hemopoiesis growth factor is a cytokine.

20. The method of claim 19, wherein said angiotensin-converting enzyme inhibitor is captopril, fentiapril, pivalpril, zefenopril, ano-alacepril, enalapril, lisinopril, benzepril, indolapril, pentopril, indalapril, ciazlapril, and fosinopril.

21. The method of claim 20, wherein said hemopoiesis growth factor is G-CSF or GM-CSF.

22. The method of claim 21, wherein the subject is undergoing chemotherapy with a cytotoxic agent, wherein said cytotoxic agent is cyclophosphamide, daunorubicine, taxol, 5-fluorouracil, adriamycin, cisplatinum, methotrexate, cytosine arabinoside, mitomycin C, prednisone, vindesine, carboplatinum, AZT, or vincristine.

23. The method of claim 21, wherein the subject is undergoing radiotherapy.

24. The method of claim 4, said method further comprises administering to said subject a hemopoiesis inhibitory factor prior to said administration of said hemopoiesis growth factor.